1 DETECTION OF BLOOD

1.1 COMBINED PHENOLPHTHALEIN-TETRAMETHYLBENZIDINE (PTMB) TEST (References 1, 2, 3, Appendix A)

1.1.1 Safety Considerations

1.1.1.1 Phenolphthalin - Caution! Avoid contact and inhalation!

1.1.1.2 Potassium hydroxide - Caution! Corrosive! Poisonous!

1.1.1.3 Tetramethylbenzidine - Caution! Harmful if swallowed, inhaled or absorbed through skin! Emits toxic fumes under fire conditions!

1.1.1.4 Glacial acetic acid - Caution! Corrosive! Flammable!

1.1.1.5 Ethanol - Caution! Flammable! Poisonous!

1.1.1.6 Oxidized zinc - Caution! Danger of spontaneous combustion if allowed to dry!

1.1.2 Materials and Equipment

1.1.2.1 Dropper bottles

1.1.2.2 Cotton swabs

1.1.2.3 Test tubes, microtiter plates, or filter paper

1.1.2.4 100 ml graduated cylinder

1.1.2.5 Weigh boats or weigh paper

1.1.2.6 Balance

1.1.2.7 Spatula

1.1.2.8 Scissors

1.1.2.9 Tweezers

1.1.2.10 Magnetic stir plate

1.1.3 Stock Solutions

1.1.3.1 Phenolphthalin Stock Solution

- 1 g Phenolphthalin
- 25 g Potassium Hydroxide (KOH)
- 100 ml Distilled water

- The above ingredients are mixed until thoroughly dissolved.

1.1.3.1.1 Storage

1.1.3.1.1.1 This colorless solution is stored under refrigeration over fresh granular zinc to keep it in the reduced form. The oxidized zinc in the bottle should not be allowed to dry (see 1.1.1.6 Safety Considerations and 1.1.3.1.3.1 Disposal).

1.1.3.1.2 Labeling

1.1.3.1.2.1 Label the bottle as Phenolphthalin Stock Solution with a lot number (the date of preparation followed by the initials of the person preparing the stock solution).
Example: Phenolphthalin Stock Solution Lot Number 100899JD was prepared by Jane Doe on October 8, 1999.

1.1.3.1.2.2 There is no expiration date (see 1.1.5.1 Minimum Standards and Controls).

1.1.3.1.3 Disposal

1.1.3.1.3.1 When the reduced phenolphthalin stock solution is depleted, cover the zinc in the bottom of the bottle completely with a solution of potassium hydroxide in distilled water (25 g KOH/100 ml dH_20).

1.1.3.1.3.2 Label the bottle with the contents and "For Disposal" and refrigerate. Notify the Safety Officer (Eastern, Northern, and Western Laboratories) or the Department Safety Coordinator (Central Laboratory) that the zinc is ready to be disposed of in accordance with Department procedures.

1.1.3.2 Tetramethylbenzidine (TMB) Stock Solution

- 10 mg Tetramethylbenzidine (TMB)
- 30 ml Glacial acetic acid
- Mix the above ingredients until thoroughly dissolved.

1.1.3.2.1 Storage

1.1.3.2.1.1 The TMB stock solution should be stored at room temperature.

1.1.3.2.2 Labeling

1.1.3.2.2.1 Label the bottle as TMB Stock Solution with a lot number (the date of preparation followed by the initials of the person preparing the stock solution).
Example: TMB Stock Solution Lot Number 100899JD was prepared by Jane Doe on October 8, 1999.

1.1.3.2.2.2 There is no expiration date (see 1.1.5.1 Minimum Standards and Controls).

1.1.3.2.3 Disposal

1.1.3.2.3.1 Dispose of the TMB stock solution and other materials contaminated with this solution as hazardous waste in accordance with Department procedures.

1.1.4 Working Solutions

- Distilled water
- Ethanol
- 3% Hydrogen peroxide
- 1:5 dilution of phenolphthalin stock solution in distilled water (1 part of the phenolphthalin stock solution diluted with 4 parts of distilled water)
- TMB stock solution

1.1.4.1 Storage

1.1.4.1.1 All bottles of working solutions are stable at room temperature.

1.1.4.2 Labeling

1.1.4.2.1 Bottles containing working solutions of ethanol and 3% hydrogen peroxide will be labeled with the contents and the lot number.

1.1.4.2.2 The bottle containing the 1:5 dilution of phenolphthalin stock solution must be labeled appropriately. If the lot number of the diluted stock solution is recorded in the reagent log book, then the bottle must be labeled with this lot number. If only the neat stock solution is recorded in the reagent log book, then the bottle must be labeled with the lot number of the neat stock solution, the date of the dilution, and the initials of the person making the dilution.

1.1.4.2.3 There is no expiration date for the working solutions (see 1.1.5.1 Minimum Standards and Controls).

1.1.5 Minimum Standards and Controls

1.1.5.1 On the day of use a positive reagent control (known bloodstain) and a negative reagent control (distilled water) must be tested to ensure that the reagents are working properly. The results of this testing must be documented in the case file.

1.1.5.2 If either control does not give the expected result, do not proceed with testing evidence samples until the problem has been resolved as demonstrated by testing another set of positive and negative reagent controls and achieving the expected results with both controls.

1.1.5.3 As a general rule a substrate control will not be tested nor is it necessary to test submitted control swabs.

NOTE: Caution should be used when testing leather and suede items since the tannins used on these products may cause a positive result in the absence of blood.

1.1.6 COMBINED PHENOLPHTHALEIN-TETRAMETHYLBENZIDINE (PTMB) TEST PROCEDURE

1.1.6.1 Gently rub a suspected stain with a cotton swab which has been moistened with distilled water or place a small cutting of the stain in a small test tube or microtiter plate, or on filter paper and moisten with distilled water if desired.

1.1.6.2 Add one drop of ethanol.

1.1.6.3 Add one drop of 1:5 dilution of phenolphthalin (i.e., the working solution of phenolphthalin).

1.1.6.4 Add one drop of 3% hydrogen peroxide.

1.1.6.5 Note any color change. An immediate pink color is expected if blood is present.

1.1.6.6 Add one drop of tetramethylbenzidine stock solution.

1.1.6.7 Note any color change. An immediate blue-green color is expected if blood is present.

1.1.6.8 Interpretation

1.1.6.8.1 Positive Reaction = Immediate pink color at 1.1.6.5, followed by immediate blue-green color at 1.1.6.7

1.1.6.8.2 Negative Reaction = No color change at 1.1.6.5, followed by no color change at 1.1.6.7

1.1.6.8.3 Inconclusive Reaction = Development of color combinations other than those specified for a positive reaction, including one test positive and the other test negative

1.1.6.9 Reporting Results

1.1.6.9.1 Report positive test results as “blood was indicated…”

1.1.6.9.2 Report negative test results as “no blood was indicated...”

1.1.6.9.3 Report inconclusive test results as “tests for blood were inconclusive…”

1.1.6.9.4 Report a negative observation as “no blood was observed on ….”

1.2 LUMINOL TEST (Reference 4, Appendix A)

1.2.1 Safety Considerations

1.2.1.1 Sodium perborate - Caution! Harmful if swallowed, inhaled or absorbed through skin!

1.2.1.2 Aminophthalhydrazide (luminol) - Caution! Irritant! Emits toxic fumes under fire conditions!

1.2.2 Materials and Equipment

1.2.2.1 Spray bottle (must contain no metal parts as the luminol reacts with some metals)

1.2.2.2 50 ml graduated cylinder

1.2.2.3 Balance

1.2.2.4 Weigh boats or weigh paper

1.2.2.5 Spatula

1.2.2.6 Ziploc bags, conical tubes, or other appropriate containers (optional)

1.2.2.7 Magnetic stir plate

1.2.3 Stock Solutions

1.2.3.1 Solution A

- 0.7 g Sodium perborate
- 50.0 ml Distilled water
- Mix above ingredients until thoroughly dissolved.
- USE IMMEDIATELY! DO NOT STORE.

1.2.3.2 Solution B

- 0.1 g Aminophthalhydrazide (luminol)
- 5.0 g Sodium carbonate
- 50.0 ml Distilled water
- Mix the above ingredients until thoroughly dissolved.
- USE IMMEDIATELY! DO NOT STORE.

NOTE: The dry chemicals in Solutions A and B can be weighed out and placed in appropriately labeled containers and stored in the dark at room temperature. Each container must be labeled with the date prepared, the initials of the person who prepared each package, and the amount of distilled water to be added. Water can be added when needed.

1.2.4 Minimum Standards and Controls

1.2.4.1 Test a positive reagent control (known bloodstain) and a negative reagent control (distilled water) to ensure that the reagents are working properly. The results of this testing must be documented in the case file.

1.2.4.2 If either control does not give the expected result, do not proceed with testing evidence samples until the problem has been resolved as demonstrated by testing another set of positive and negative reagent controls and achieving the expected results with both controls.

1.2.4.3 If the results of the test are positive, as a general rule a substrate control will not be tested.

1.2.5 LUMINOL PROCEDURE

1.2.5.1 **When the test is ready to be conducted,** mix equal parts of solutions A and B and place in a sprayer bottle.

1.2.5.2 **Under darkened conditions**, immediately after mixing equal parts of solutions A and B, spray the positive and negative controls to ensure that the reagents are working properly. If both controls give the expected results, proceed with spraying the area of interest. Document results in the case file.

1.2.5.3 Areas containing blood will luminesce immediately.

1.2.5.4 Mark luminescent areas for subsequent testing with the Combined Phenolphthalein-Tetramethylbenzidine Test.

1.2.5.5 Interpretation

1.2.5.5.1 Positive Reaction = Immediate luminescence

1.2.5.5.2 Negative Reaction = No luminescence

1.2.5.5.3 Inconclusive Reaction = Slow and/or weak luminescence

1.3 BLUESTAR® FORENSIC TEST

BlueStar® Forensic Test Kit information available at http://www.bluestar-forensic.com

1.3.1 Safety Considerations

1.3.1.1 Beige Tablet - Caution! Avoid contact with skin and eyes, gloves must be worn

1.3.1.2 White Tablet - Caution! Avoid contact with skin and eyes, gloves must be worn

1.3.2 Materials and Equipment

1.3.2.1 Spray bottle (must contain no metal parts, chemicals may reacts with some metals)

1.3.2.2 100 ml graduated cylinder

1.3.3 Stock Solutions

1.3.3.1 BlueStar® Forensic Working Solution

- White and beige BlueStar® Forensic tablets

- 125 ml Distilled water
- Dissolve white and beige tablets in water
- USE IMMEDIATELY! DO NOT STORE.

1.3.4 Minimum Standards and Controls

1.3.4.1 Test a positive reagent control (known bloodstain) and a negative reagent control (distilled water) to ensure that the reagents are working properly. The results of this testing must be documented in the case file.

1.3.4.2 If either control does not give the expected result, do not proceed with testing evidence samples until the problem has been resolved as demonstrated by testing another set of positive and negative reagent controls and achieving the expected results with both controls.

1.3.5 BLUESTAR® FORENSIC PROCEDURE

1.3.5.1 **Under darkened conditions**, immediately after dissolving the tablets in water, spray the positive and negative controls to ensure that the reagents are working properly. If both controls give the expected results, proceed with spraying the area of interest. Document results in the case file.

1.3.5.2 Areas containing blood will luminesce immediately.

1.3.5.3 Mark luminescent areas for subsequent testing with the Combined Phenolphthalein-Tetramethylbenzidine Test.

1.3.5.4 Interpretation

1.3.5.4.1 Positive Reaction = Immediate luminescence

1.3.5.4.2 Negative Reaction = No luminescence

1.3.5.4.3 Inconclusive Reaction = Slow and/or weak luminescence

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